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Seed Germination Response to Temperature and Salinity Stress in *Lilium longiflorum* and *L. formosanum*

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Seed germination response to different degree of thermal and salinity treatments for two closely related, but ecologically distinct bulbous species, *Lilium longiflorum* and *L. formosanum* was investigated, in order to clarify optimum temperature for seed germination and whether the tolerance to salinity stress is different between the species. Among seven constant thermal treatments for seeds collected from two natural populations of *L. longiflorum* and one of *L. formosanum*, treatments of 25 and 30 °C significantly reduced the final germination percentage approximately less than 80 and 10%, respectively, of the treatments below 20 °C. Treatment exposed to 17.5 or 20 °C allowed the maximum germination rate and percentage, irrespective of the seed source populations. By exposure to 1 and 2% NaCl at 17.5 °C, 97 and 93% of the seeds, respectively, were germinated in *L. formosanum*. The values were comparable to or greater than the highest values obtained from *L. longiflorum*. Both the species could little germinate in 3% NaCl treatment. The results indicate that *L. formosanum* is not physiologically distinct from *L. longiflorum* in terms of salinity tolerance. It is concluded that salinity stress, which is one of the major abiotic environmental stresses specific to habitats of *L. longiflorum*, cannot work as a factor of habitat isolation between the two species.

INTRODUCTION

Lilium longiflorum and *L. formosanum* are Asiatic bulbous species very useful as ornamental plant resources. They have been considered to be closely related species as presumed from easy production of horticultural interspecific hybrid, *L.* x *formolongi* by artificial hybridization (Okazaki, 1996) and genetic similarity in ITS sequences of rDNA (Dubouzet and Shinoda, 1999). More recently, study on genetic structures of natural populations of the two species has indicated that the *L. formosanum* is highly likely a recent local derivative from *L. longiflorum* (Hiramatsu *et al.*, 2001a).

Both the species are subtropical to temperate insular plants (Wilson, 1925; McRae, 1998). Distribution ranges of the two species are overlapping in the mainland of Taiwan (Shii, 1983). However, habitats where natural populations have been established are remarkably different between *L. longiflorum* and *L. formosanum*, and the species have never been found to be growing sympatrically under natural condition (Hiramatsu *et al.*,

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2001b). *L. longiflorum* is mainly growing on well–lit grassy fields often dominated with *Zoysia* sp. or on rocky cliffs, very close to the sea. Such habitats presumably give plants salinity stress. On the other hand, *L. formosanum* preferably has developed populations in the inland vegetation affected by human and natural disturbance in the margins of arable lands and forests, and on the mountain slopes and cliffs. These habitats are generally considered to be much free from salinity stress.

The environmental factors typical of such species–specific habitats may be closely concerned with adaptive strategies acquired during establishment and/or persistence of the species. We thus hypothesized that salinity stress is one of the major environmental factors differentiating those species' habitats. Salinity is a serious problem encountered by seeds in soils and affects greatly on seedling recruitment (Karssen and Hilhorst, 1992). The major objective in the present study is to estimate effect of salinity stress on germination of *L. longiflorum* and *L. formosanum*.

As for seed germination, temperature is a well–known principle factor. There are, however, few explicit studies of which temperature is the most effective for promoting seed germination of *Lilium* species. The present study also addressed the effect of temperature on seed germination.

MATERIALS AND METHODS

Seeds were collected from two populations of *L. longiflorum* located in a coastal area of Kume Shima Island (LKU) in the Ryukyu Archipelago and of Pitouchiao (LPI) in the mainland of Taiwan, and one population of *L. formosanum* located on a mountainous area of Lishan (FLI) in the mainland of Taiwan. They were air–dried at room temperature for maximally nine months until the experiment.

Seven treatments with constant temperature at 5, 10, 15, 17.5, 20, 25 and 30 °C under a dark condition were applied to the seeds using incubators. Three replications of 50 to 60 seeds were subjected to each treatment. Seeds were placed on 50 ml of vermiculite in 9 cm petri dishes, and then approximately 27 ml of deionized distilled water was added. Agrimycine®–100 (18.8% streptomycin sulfate and 1.5% oxytetracycline, Pfizer Co.) was added to the germination medium at the concentration of 0.1 gl^{-1} in order to prevent proliferation of bacteria. Germination of each seed was determined by verification of protrusion of the radicle every day.

Sensitivity to salinity during germination was determined at 0, 1, 2 and 3% of sodium chloride (NaCl) solution at 17.5 °C under a dark condition.

RESULTS AND DISCUSSION

Germination responses to thermal treatments

Final germination percentage was nearly 100% at 10–20 °C, whereas it was significantly decreased as temperature was raised above 20 °C (Fig. 1). The treatment by the highest temperature of 30 °C remarkably suppressed the germination percentage as low as 10% or below. The highest final germination frequency and the earliest germination date when 50% of final germination percentage was observed were obtained at 17.5 or 20 °C irrespective of seed source populations. The results indicate that the germination of

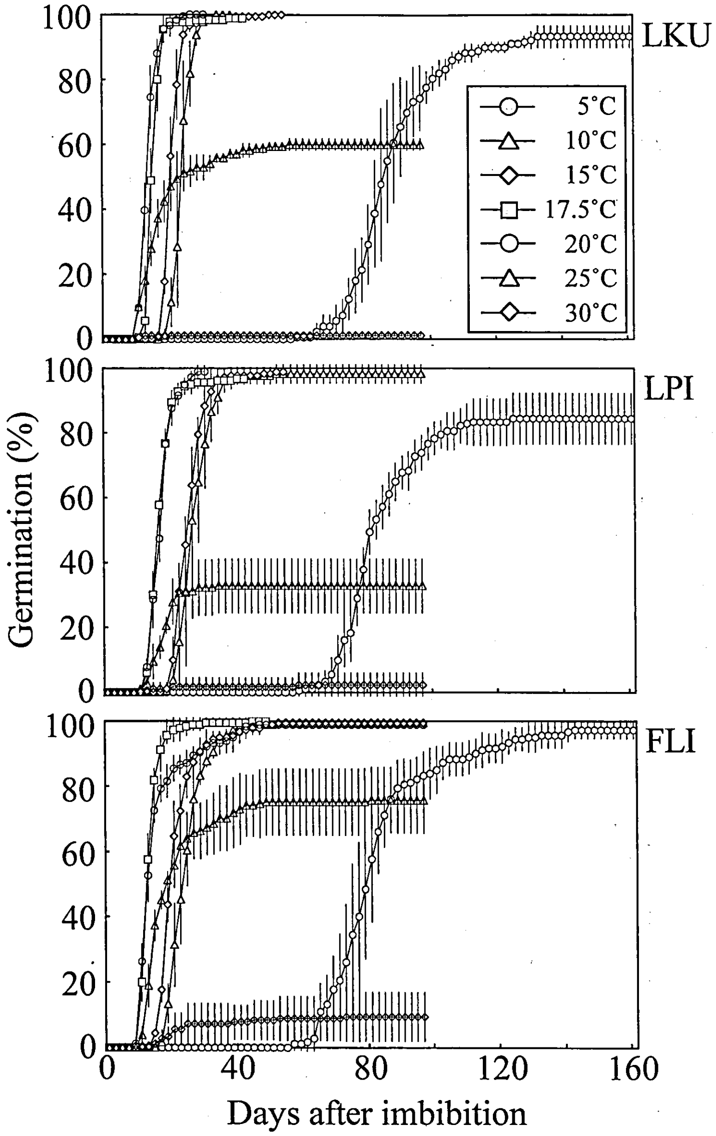

Fig. 1. Germination responses of seeds harvested from two natural populations of *Lilium longiflorum* and one of *L. formosanum* to seven different levels of constant temperature. Vertical bars of each plot are standard deviations of three replications. LKU, LPI, and FLI, respectively, indicates the seed source population of *L. longiflorum* from Kume Shima Island in the Ryukyu Archipelago, from Pitouchiao in the mainland of Taiwan, and that of *L. formosanum* from Lishan in the mainland of Taiwan.

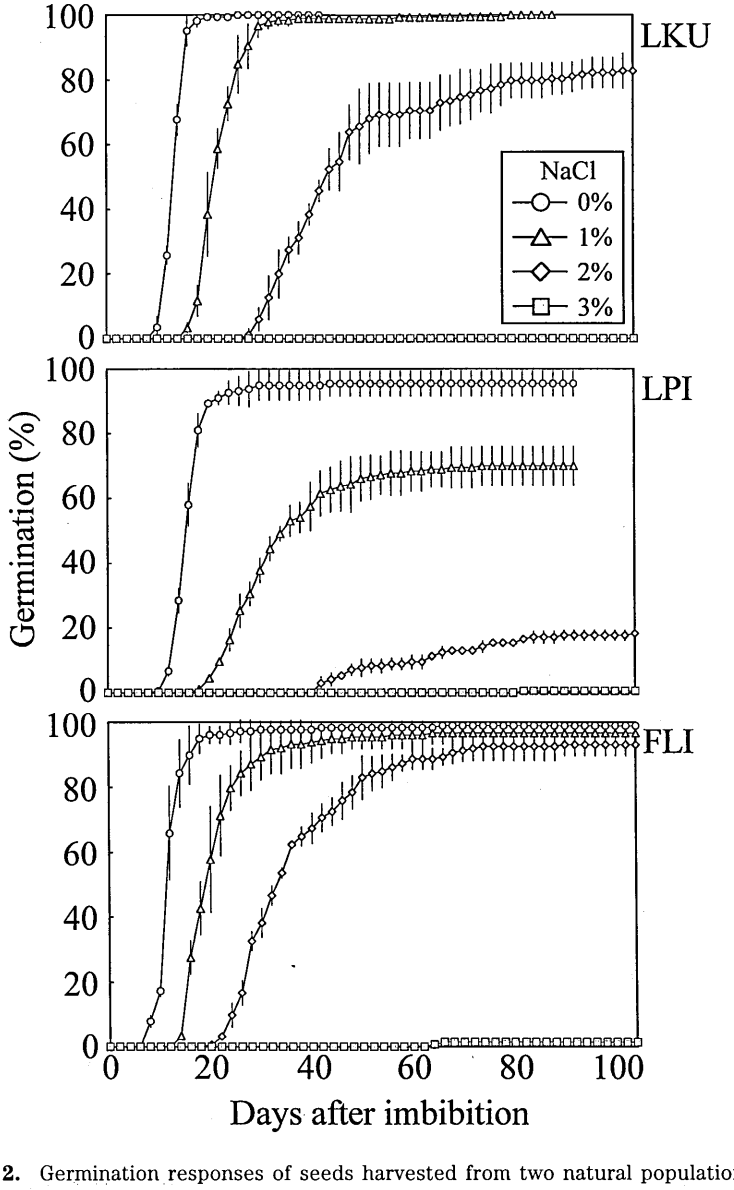

Fig. 2. Germination responses of seeds harvested from two natural populations of *Lilium longiflorum* and one of *L. formosanum* to four different levels of NaCl concentration at 17.5 °C. Vertical bars of each plot are standard deviations of three replications. Abbreviations of population names are the same as in Fig. 1.

L. longiflorum and *L. formosanum* is optimally promoted in the range of 17.5–20 °C, and it is suppressed above 20 °C.

Germination responses to salinity treatments

Both the final germination percentage and the germination rate decreased with increasing salinity, and little germination was observed in 3% NaCl solution for all populations (Fig. 2). Decreasing patterns of the germination rate and final germination percentage with increasing salinity were significantly different among populations studied. For example, the final germination percentage of population LPI was decreased to 18% in 2% NaCl solution, whereas that of population LKU and FLI, respectively was as high as approximately 83 and 93% in the same salinity treatment. However, even for population LPI, high germination percentage (70%) was exhibited in 1% NaCl solution.

These results indicate that both the species essentially possessed relatively as high tolerance to salinity in their germination as halophytes, in which the delay in germination and final reduction in total number of germinated seeds may not occur until concentrations above 1% NaCl (Unger, 1978), and may imply that environmental factors other than the soil salinity stress are responsible for the reason why *L. longiflorum* and *L. formosanum* do not grow sympatrically. Alternatively, Dubouzet and Shinoda (1999) attributed the reason why *L. longiflorum* is preferably grown on coral limestone to its tolerance to alkaline soil and they also stated that the species grows poorly on acidic soils. Their hypothesis may be significant to be verified.

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